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14. ABSTRACT

Work on sleep at Brandeis focuses on *Drosophila melanogaster* as well as the more traditional rodent models. The *Drosophila* works aims to exploit the genetic advantages of this organism yet still learn about aspects of sleep relevant to humans. The major finding has been that the human therapeutic Carbamazepine is a potent sleep-deprivation agent in flies. Current data indicate that its effects are mediated through the *Rdl* $GABA_A$ receptor, which has implications for the role of this drug in humans. One of the rodent laboratories is focused on the regulation of sleep and waking in the basal forebrain. The goal is to identify gene expression changes in its cholinergic neuronal subset, and specific neuron purification has been accomplished. Another rodent laboratory is studying the effects of sleep deprivation on the intrinsic electrophysiology and gene expression properties of neocortical neurons. Interesting changes in firing properties of layer 5 pyramidal neurons have been observed, and gene expression assays from these cells are underway. The final two projects involve the role of sleep in homeostatic plasticity and fear conditioning. These are being done both in vivo, in freely behaving animals, and "ex vivo, in cortical slices after sleep deprivation or training.

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General Introduction

There were six PIs at Brandeis with a common interest in sleep. During the past year, we have added two additional investigators, Drs. Agar and Hong. Dr. Agar is a specialist in mass spectrometry and Dr. Hong a specialist in bioinformatics. As their reports detail, we anticipate fruitful collaborations between the original six and these two new laboratories. They will open up new research avenues for the rest of us.

Although the original six investigators run autonomous research programs with individual reports, every research program has interactions with one or more other of the other laboratories. This is a characteristic of Brandeis, i.e., close and productive inter-laboratory collaborations. Indeed, it is anticipated that all publications on sleep from Brandeis will reflect collaborations between two or more laboratories. We are a highly interactive research community.

Finally, I emphasize that the sleep community at Brandeis is relatively young. Most of us have no long-term track record in this field and have become interested in sleep only in the past few years. So we all began this work de novo. As a consequence, the group is only now entering a mature phase. As such, much of the work initiated over the past almost two years should reach fruition (i.e., publication) during the next year or two. Please see the individual reports for more detail.

Each Aim in the original and supplemental Statements of Work is being pursued by an individual laboratory at Brandeis University. As such the introduction, body, key research accomplishments, reportable outcomes, conclusions, and references for each aim are reported as separate sections by the PI of the laboratory who is pursuing the aim. There are also several inter-lab collaborations, which should be obvious from the individual lab aims. They are presented in the chronological order they were funded. Aims 1-6 from the original Statement of Work, funded 01/15/04 to 01/14/07 and in the second year of research, are followed by the Mass Spec and Bioinformatics Aims proposed in the supplementary Statement of Work, funded 01/15/05 to 01/14/08 and in the first year of research.

Aim 1: To identify the molecular targets of the wakefulness-promoting drug modafinil using forward genetics in *Drosophila* (Griffith)

Introduction

Drosophila exhibit a sleep-like state that shares several characteristics of human sleep (Hendricks et al., 2000; Shaw et al., 2000). Flies therefore potentially provide a useful tool with which to dissect the genetic drive for sleep. Flies entrained in 12:12 light:dark (L:D) cycles have sustained periods of activity and rest; rest occurring primarily during the dark period for female flies (Shaw et al., 2000). The effects of many sleep modulating compounds are thought to be conserved. Our initial intention was to investigate the effects of Modafinil on fly sleep and to use forward genetics to identify its targets. Unfortunately, we have been unable to reproduce the published effects of this drug (Hendricks et al., 2003) and we have reformulated our goals to 1) identify the molecular mechanism of carbamazepine's sleep disrupting effects 2) map the circuitry that controls sleep. These experiments are being carried out in collaboration with the Rosbash lab.

Body

1) Molecular mechanism of Carbamazepine (CBZ) effects on sleep.

In humans, CBZ is used to treat epilepsy, trigeminal neuralgia, and bipolar disorder. Our progress in understanding the role of the drug in flies is described below in Aim 2. A salient feature of these results is that the *Rdl* $GABA_A$ receptor is a direct target of CBZ and that this interaction can largely account for the behavioral effects of the drug. The effects of CBZ on Rdl appear to be mediated by the TM2 domain, which in human $GABA_A$ receptors is believed to be an effector for drug action or possibly to provide a binding site for drugs. Recordings made from oocytes expressing wild type human $GABA_A$ $\square_1\square_2\square_{2S}$ receptor respond to CBZ with an increase in current amplitude, consistent with CBZ's human efficacy as an anticonvulsant. Mutations made in the region analogous to the fly Rdl point mutant block this effect. The difference in CBZ's effects between human and fly and the ability of similar point mutations to block them support the idea that TM2 is an important region and that subtle differences in this region are responsible for the divergent CBZ effects in these two species. We plan to make fly/human chimeric channels over this small region to investigate the mechanism of this difference. These studies may provide 1) clues to how TM2 gates this channel; 2) mutant channels that respond with opposite sign that can be used in transgenic studies to map sleep circuits; 3) a platform on which to screen for vigilance-promoting CBZ analogs.

2) Sleep circuits.

Circadian and homeostatic circuitries are thought to underlie the sleep-like state in flies. By using cell-specific ablation, it has been shown that the timing of locomotor activity is driven in distinct groups of clock neurons (Stoleru et al., 2004). Using genetic ablation in *cry*-GAL4;UAS-*hid* transgenic animals

(Stoleru et al., 2004) under L:D and D:D conditions we found that the clock may act to modulate the pattern of sleep but clock neurons are not part of the circuit that generates the sleep state.

To further investigate the mechanisms that underlie modulation of sleep by the clock we expressed transgenes encoding the *Shaw* potassium channel or *Shaw* RNAi in subsets of the clock circuit. Expression of *Shaw*, which is a leak channel, would be expected to block firing while expression of *Shaw* RNAi will enhance firing in cells whose resting potential is regulated by endogenous Shaw (Hodge et al., 2005). Animals expressing *tim*-GAL4;UAS-*Shaw* or *tim*-GAL4;UAS-*Shaw/pdf*-GAL80 fail to suppress sleep during the subjective day in D:D, but not in L:D. Flies expressing *tim*-GAL4;UAS-*ShawRNAi* have more daytime sleep in both L:D and D:D. This effect is suppressed by *pdf*-GAL80. These results suggest that the activity of LN_ds is necessary for suppression of sleep during the day when light, which appears to activate a clock-independent suppression pathway, is not available. The RNAi experiment is consistent with LN_v having an active role in modulating the effects of LN_d activity.

The robust effects of CBZ on sleep suggest that neurons that express the *Rdl* $GABA_A$ receptor may be critical for either the generation of sleep or the output of the sleep circuit. To gain entrée into this circuit, we have generated transgenic flies that express either the wild type (RdlWT) or CBZ-resistant mutant (RdlA302S). Expression of RdlWT using a panneural GAL4 line causes an increase in sensitivity to CBZ, while expression of RdlA302S blocks the effects of the drug. These results are unexpected, but may indicate that expression of these receptors in inappropriate cell types can have dominant or developmental effects. We are currently expressing these constructs in more limited regions to investigate these possibilities.

Key Research Accomplishments

- circadian clock neurons do not generate sleep
- CBZ enhances locomotor activity by stabilizing the desensitized state of the *Rdl* $GABA_A$ receptor and this is mediated by TM2
-human $GABA_A$ receptors show a similar block of CBZ effects with TM2 mutation
-modulation of excitability in clock cells identifies LN_ds as controllers of daytime sleep suppression.

Reportable Outcomes

Agosto, J.L., Choi, J.C.K., Griffith, L.C. and Rosbash, M. (2006) "Carbamazepine affects *Drosophila* sleep by altering $GABA_A$ receptor gating", *Submitted*

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Stoleru, D., Peng, Y., Agosto, J., and Rosbash, M. (2004). Coupled oscillators control morning and evening locomotor behaviour of Drosophila. *Nature* **431,** 862-8.

Aim 2: To determine the role played by *Drosophila* clock neurons in regulating sleep and to identify other groups of neurons in the *Drosophila* brain involved in regulating sleep and wakefulness (Rosbash)

Introduction

As described above, *Drosophila* exhibit a sleep-like state that shares several characteristics of human sleep (Hendricks et al., 2000; Shaw et al., 2000). Flies therefore potentially provide a useful tool with which to dissect the genetic drive for as well as the importance of conserved molecules to sleep. Flies entrained in 12:12 light:dark (L:D) cycles show sustained periods of activity and rest; rest occurring primarily although not exclusively during the dark period (Shaw et al., 2000). The effects and targets of many sleep modulating compounds are thought to be conserved. Our initial intention was to screen known human therapeutics, especially those with unknown or poorly defined targets, for their effects on fly behavior. The most striking result we obtained was the almost complete elimination of fly sleep with the drug carbamazepine. We are pursuing experiments to define its mechanism of action as well as using it as a tool to investigate aspects of fly sleep. These experiments have been carried out in collaboration with the Griffith lab.

Body

1) Molecular mechanism of Carbamazepine (CBZ) effects on sleep.

In humans, CBZ is used to treat epilepsy, trigeminal neuralgia, bipolar disorder. The basis of these actions is believed to be due to interaction of the drug with Na^+ channels and $GABA_A$ receptors. In flies, we have discovered that CBZ causes both an increase in locomotor behavior and a decrease in sleep. The increase in activity is completely accounted for by the decrease in sleep/rest; This effect appears to be mediated through the *Rdl* $GABA_A$ receptor since *Rdl* point mutants that are resistant to dieldrin (an insecticide) are also resistant to CBZ. Moreover, flies with reduced levels of Rdl protein (*Df*/+ genotype) are hypersensitive to the drug. To determine if CBZ interacts directly with the receptor and how it affects GABA responses, we have recorded from *Xenopus* oocytes expressing wild type and mutant Rdl. Rdl in this system gives a robust, but quickly desensitizing response to GABA that can be measured electrophysiologically. Application of CBZ selectively stabilizes the desensitized state of the receptor. The point mutant that blocks CBZ effects *in vivo* does not show this type of stabilization. These results suggest that the *Rdl* $GABA_A$ receptor is a direct target of CBZ and that this interaction can largely account for the behavioral effects of the drug. These initial behavioral and electrophysiological results have been submitted for publication.

2) Sleep circuits and mammals.

To further investigate the contribution of different brain regions to sleep regulation, we are keen to find the location(s) that contain the predicted Rdl-expressing inhibitory neurons. We are in the process of testing various candidate brain regions. The prediction is that their ablation should alter CBZ-sensitivity. We are also interested in moving some of these experiments to the mouse, which we plan to do during the next year. The prediction is that a "knock-in" of the fly mutation should make a mouse less CBZ-sensitive, if this $GABA_A$ subunit is also a major CBZ-target in mammals.

Key Research Accomplishments

- Discovery that CBZ inhibits fly sleep and identification of putative CBZ target
- CBZ enhances locomotor activity by stabilizing the desensitized state of the *Rdl* $GABA_A$ receptor, which is mediated by TM2 domain

Reportable Outcomes

Agosto, J.L., Choi, J.C.K., Griffith, L.C. and Rosbash, M. (2006) "Carbamazepine affects *Drosophila* sleep by altering $GABA_A$ receptor gating", *Submitted*

References

Hendricks, J. C., Finn, S. M., Panckeri, K. A., Chavkin, J., Williams, J. A., Sehgal, A., and Pack, A. I. (2000). Rest in Drosophila is a sleep-like state. *Neuron* **25,** 129-38.

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Stoleru, D., Peng, Y., Agosto, J., and Rosbash, M. (2004). Coupled oscillators control morning and evening locomotor behaviour of Drosophila. *Nature* **431,** 862-8.

Aim 3: Regulation of gene expression during sleeping and waking (Birren).

Introduction

Mammalian sleep regulation relies on the coordination of multiple neurotransmitter systems in different brain regions. The goal of this project is to use gene expression profiling techniques to understand how different neuron types within the mammalian basal forebrain contribute to sleep regulation pathways. The basal forebrain provides contains two distinct neuron types, both of which have been linked to sleep regulation. Basal forebrain cholinergic neurons project to cortical and hippocampal targets and play a major role in the control of arousal, attention and in the development and modulatation of cortical circuits (Berger-Sweeney et al., 2001). These neurons have increased activity during waking periods and REM sleep when compared to activity levels during non-REM sleep (Detari et al., 1984; Detari and Vanderwolf, 1987; Szymusiak and McGinty, 1986). Thus, these neurons may contribute to cortical activity associated with the waking state. GABAergic neurons of the basal forebrain also project to cortical targets as well as making local connections within the basal forebrain. In contrast to the cholinergic neurons of the basal forebrain, GABAergic neurons show decreased activity during REM sleep (Pollock and Mistlberger, 2003; Sanford et al., 2003). GABAergic transmission may have inhibitory effects on cholinergic transmission, suggesting that these systems use reciprocol patterns of activity to regulate sleep cycles (Vazquez and Baghdoyan, 2003). The local interactions of this system within the basal forebrain and the importance of this system in sleep regulation makes the basal forebrain an excellent candidate for investigation of the regulatory pathways underlying sleep-wake transitions. We are therefore investigating sleep cycle associated patterns of gene expression in the basal forebrain.

Body

In previous work on this project we used PCR and gene arrays to demonstrate that sleep deprivation resulted in altered gene expression in basal forebrain and cortical neurons, including increases in activity-dependent genes such as brain-derived neurotrophic factor (BDNF) and homer. These changes are consistent with changes in gene expression seen in other studies (Cirelli et al., 2004; Cirelli and Tononi, 2000; Nelson et al., 2004). One questioned raised by all of these studies on sleep-dependent regulation of gene expression concerns the neuronal specificity of the regulation. If, as in the case of the basal forebrain, some neuron populations are increasing activity during sleep or waking, and other neurons in the same region are decreasing activity we could potentially be losing important information. Thus, sleep promoting gene expression changes and gene expression associated with the waking state could show temporal overlap in the different populations. Further, the identity of genes that are reciprocally regulated in the two popoulation would be lost in gene array approaches that use total basal forebrain RNA as a starting material. We have therefore turned our attention to the identification and isolation of individual neuron types within the basal forebrain with the goal of using purified populations to define sleep dependent changes specific to cholinergic neurons.

1) Identification of cholinergic neurons in key basal forebrain nuclei. We have use antibodies raised against choline acetyltransferase (ChAT) to identify cholinergic neurons in vibrotome sections of several basal forebrain nuclei (Fig. 1). This staining procedure clearly delineates the medial septum (MS), the vertical limb of the diagonal band of Broca (VDB), and the horizontal limb of the diagonal band of Broca (HDB). Importantly, these images demonstrate that additional cholinergic neurons (for instance in the nucleus accumbens) are well separated from basal forebrain regions that we will use cell isolations.

2) p75 as a specific marker for basal forebrain cholinergic neurons. While ChAT staining provides unambiguous marker for the cholinergic population, its intracellular localization precludes its use for cell isolation. The p75 neurotrophin receptor was reported to be selectively expressed in cholinergic neurons in this region (Tremere et al., 2000). Antibodies directed against the extracellular domain of p75 are available, suggesting the use of these antibodies in cell isolation procedures. We therefore carried out double immunolabeling of ChAT and p75 to assess the suitability of p75 expression for selective isolations of cholinergic neurons from basal forebrain. We found almost complete co-expression of ChAT and p75 in the MS, VDB and HDB, the major basal forebrain nuclei that we plan to use in our microarray screens (Fig. 2). Interestingly, nearby cholinergic neurons in the nucleus accumbens were negative for p75 expression, further confirming the specificity of p75 staining.

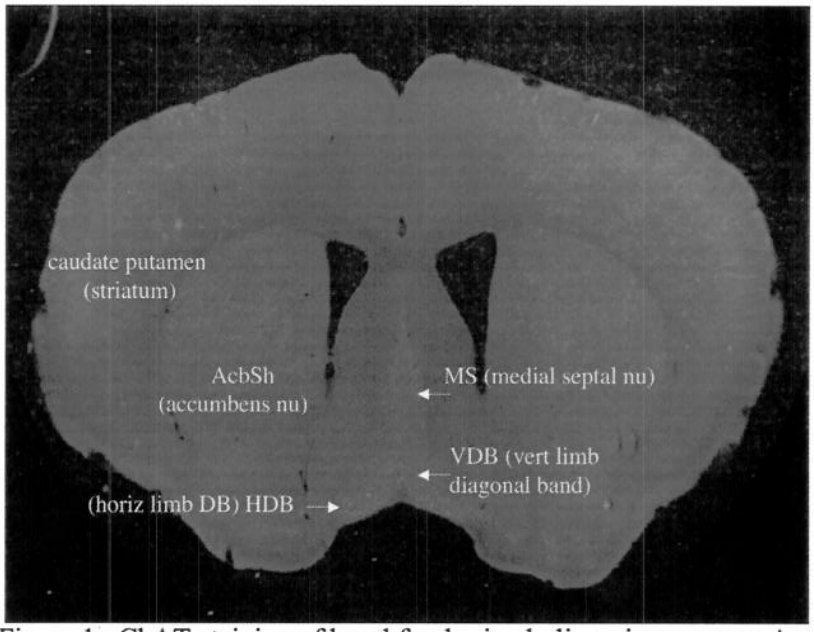

Figure 1. ChAT staining of basal forebrain cholinergic neurons. A 50 μm vibrotome section through the basal forebrain of an adult mouse was stained with a ChAT-specific antibody. Cells were labeled in basal forebrain nuclei (medial septum-MS; vertical and horizontal limbs of the diagonal band of Broca (VDB and HDB).

3) Fluorescence-activated cell sorting of p75-positive cholinergic neurons. We carried out dissections of adult mouse basal forebrain that included the MS, VDB, and HDB regions. Fresh 50 μm sections were cut from adult mouse brain at an axial level to include the basal forebrain nuclei. Microdissection was performed to obtain the region containing the MS, VDB and HDB and to exclude the neocortex, nucleus accumbens and caudate putamen. The tissue was stained with an anti-p75 antibody directly conjugated to the Cy3 fluorochrome (Hartig et al., 2002), followed by protease digestion and sorting on a FACSAria Fluorescence-activated cell sorter.

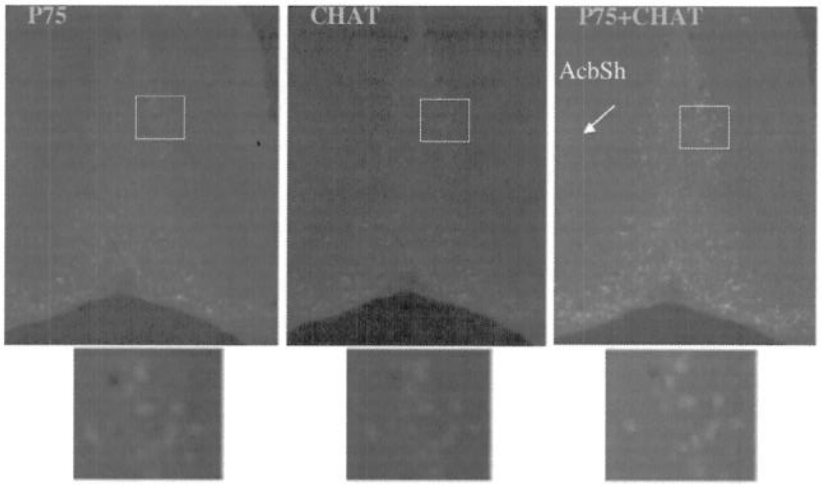

Figure 2. Double labeling of adult mouse sections of basal forebrain stained with antibodies for p75 (left) or ChAT (middle). The left panel shows a composite image. The arrow shows ChAT-positive, p75-negative cells in the nucleus accumbens (AcbSh). Lower panels show details of the areas in the white boxes, above.

Approximately 1-2% of the cells were p75-positive upon initial analysis of the cell population. Following sorting we obtained a purity of greater than 95% p75-labeled cells (data not shown).

We have now carried out this isolation several times on control animals and are able to obtain in excess of 1000 cholinergic neurons per animal. This is a sufficient number of cells to generate probes for microarray screens. We are currently using the p75-sorted cells to generate cholinergic neuron-specific mRNA that will be used for confirmatory analysis of gene expression. In collaboration with Dr. Sacha Nelson's laborabory we will apply this isolation technique to control and sleep deprived animals to define cholinergic neuron-specific patterns of gene expression associated with sleep deprivation in the basal forebrain.

Key Research Accomplishments

-Delinated regions of cholinergic neurons within mouse basal forebrain nuclei.
-Identified p75 as a specific marker of cholinergic neurons within the basal forebrain.
-Developed microdissection and dissociation procedures for the isolation of basal forebrain nuclei.
-Successfully used high through-put fluorescence-activated cell sorting for the isolation of purified populations of cholinergic basal forebrain neurons.

Reportable Outcomes

-none

Conclusion

We had previously demonstrated progress in establishing sleep deprivation protocols and in the analysis of gene expression using PCR and DNA microarray-based approaches. The next key task was to establish our ability to examine gene expression in specified populations of basal forebrain neurons. We have now accomplished this goal by defining the cholinergic populations within basal forebrain nuclei, demonstrating that the cholinergic neurons are selectively marked by the p75 neurotrophin receptor, and using a directly conjugated p75 antibody to obtain highly purified basal forebrain cholinergic neurons. We will now go on to bring these techniques together to examine gene regulation in the cholinergic neurons. We will generate probes from purified cholinergic neurons obtained from sleep deprived and control mice and use them to screen DNA microarrays. The basal forebrain is a key regulator of sleep patterns and this study will shed light on the specific role of these cholinergic neurons in the regulation of sleeping and waking.

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Aim 4: Effects of sleep deprivation on neuronal physiology and gene expression (Nelson).

Introduction

The primary objective of this project is to identify the effects of sleep deprivation (SD) on the intrinsic electrophysiology and gene expression of neocortical neruons. In the last report we discussed the setting up of the "Disk-Over-Water"[1] apparatus to study the effect of sleep deprivation on neuronal physiology and gene-expression. Since then we have made further modifications to the sleep deprivation technique. We have made an initial set of discoveries and are now following these up with additional studies.

Body

We observed that control animals were not able to synchronize their sleep to the epochs when the experimental mouse was awake (and the disk was not rotating) and consequently also got moderately sleep-deprived. We therefore decided to run the experiment with the control mouse separately on an identical setup. The control mouse is given the same amount of physical activity by rotation of the disk but exclusively when it is awake. (Sleep state is monitored with EEG and EMG using algorithms that have been calibrated with video monitoring). With the new protocol, the experimental mice lose 70-80% of their sleep, while the control mice lose ~30%. Furthermore, the experimental mice are not able to enter deep sleep (low EEG power in delta band) before they are woken up by disk rotation.

We carried out whole-cell recordings from fluorescently labeled layer 5 pyramidal neurons of the primary motor cortex slices from experimental and control mice (YFP-H: mouse line)[2]. Our initial assay of spontaneous AP (action-potential) firing showed a variable decrease in firing frequency in the sleep-deprived mice (Control = 0.4 +/- 0.19 Hz, Sleep-deprived = 0.16 +/- 0.07) . To find out whether the difference in spontaneous activity was due to different synaptic activity/connections or due to difference in intrinsic properties, we measured neuronal firing in response to different levels of current injection in the presence of synaptic blockers. We observed that neurons from sleep-deprived animals fired at lower frequencies and had higher minimum current thresholds for evoking firing. The RMP (resting membrane potential) and voltage threshold for evoking firing were not significantly different. However, we observed that the total membrane conductance (measured at RMP) was higher in sleep deprived mice than in control mice (Control = 98 +/-5 S/F, Sleep-deprived = 124 +/- 8 S/F; conductance normalized by capacitance). We are currently carrying out experiments to determine the underlying conductance that is affected by sleep-deprivation. These results have been presented at the annual Society for Neuroscience Meeting and were selected for presentation in the "Neuroscience in Sleep and Circadian Biology DataBlitz", an ancillary event of the Annual Meeting organized by the NIH National Center on Sleep Disorders Research, and the NIH Sleep Disorders Research Advisory Board.

We have also carried out preliminary cDNA micro-array hybridization experiments to find genes that are differentially regulated by SD. A number of transcripts appear to be differentially up- or down-regulated by SD. One intriguing lead, given the physiology results, is a potassium channel, KCNQ5, which is upregulated in the SD animals. We are currently attempting to confirm this result by measuring channel protein expression using antibodies and by measuring channel function electrophysiologically. We are also performing real-time PCR experiments to confirm altered expression of other genes following SD.

Key Research Accomplishments

- Improved DOW sleep deprivation protocol to minimize deprivation of control animals while maximizing deprivation of experimental animals

- Identified physiological consequences of deprivation on firing properties of layer 5 pyramidal neurons in mouse motor cortex.
- Carried out initial RNA isolation, amplification and hybridization experiements from cortical layer 5 pyramidal neurons for analysis of deprivation-induced changes in cell-type specific gene expression

Reportable outcomes

The following previously reported manuscript has now been published. This work was primarily supported by other funding sources, but was also partially supported by the present grant as it represented a necessary step in perfecting the gene expression assays to be used in carrying out the research goals.

- Sugino K, Hempel CM, Miller M, Hattox AM, Shapiro P, Wu C, Huang ZH and Nelson SB (2005) Molecular taxonomy of major neuronal classes in the adult mouse forebrain. Nat. Neurosci. 9:99-107 (see also News&Views p. 10-12).

The following abstract reports on our progress towards understanding the elctrophysiological consequences of sleep deprivation on cortical function in mice.

- Abstract: Taneja, P., et al., *Effects of sleep deprivation on electrophysiological properties and gene expression in mouse neocortex.* Abstract Viewer/Itinerary Planner, Washington, DC: Society for Neuroscience, Program No. 500.13, 2005 (Online).

Conclusion

We have identified a reproducible physiological change in the firing properties of a major class of output neuron of the cerebral cortex in mice. We have carried out initial studies of deprivation-induced changes in gene expression in these neurons. Further work will try to address how the physiological and molecular changes are related.

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2. Feng, G., et al., *Imaging neuronal subsets in transgenic mice expressing multiple spectral variants of GFP.* Neuron, 2000. **28**(1): p. 41-51..

Aim 5: Role of Sleep in Homeostatic Plasticity (Turrigiano).

Introduction

The major goals of this Aim, which is being done in collaboration with Don Katz and Sacha Nelson, is to examine the role of sleep in homeostatic cortical plasticity. We are taking two approaches: one, to examine this in freely behaving animals using chronically implanted electrode arrays, and an "ex vivo" approach in which animals are first sleep deprived and then brain slices are made to examine changes in cortical microcircuitry. During the third funding period we will continue to develop the necessary methodology to study this phenomenon in the intact animal, and will continue with our ex vivo experiments which are now yielding interesting results.

In vivo approach:

One of the problems that has limited awake-behaving recordings in the superficial layers (II/III-IV) of the neocortex is the dimpling of brain tissue when electrodes are inserted during surgery. The tissue bends under the pressure due to the size of the bundles of wires (25 μm in diameter) and the electrode array designs that have been used in most experiments of this kind. When the tissue recovers from the dimpling the electrodes are already positioned in deep cortical layer (V-VI) and the superficial ones have been damaged, so that the wires cannot be repositioned closer to the surface. Another issue that we needed to solve is that standard electrode implants require removal of the dura mater above the area of interest. This procedure too damages the superficial portion of the cortex.

Recently Krupa and collaborators designed new electrodes arrays specifically to overcome these problems in adult rats (Krupa et al 2004). The arrays are composed of 16 wires each and limit the dimpling of brain surface because only the wires are lowered in the cortex without the need of support cannulae in the tissue. The wires are spaced about 200 μm in the wire driving bulb, are thinner (only 12 μm) and have high impedance (2-3 MΩ). This allows recording of activity from several non-overlapping neurons. The implant is performed without removing the dura mater. Instead small current pulses are delivered through each individual wire to generate a small hole for the wire to penetrate.

We have imported this technology into the lab and are attempting to adapt it to our purposes. Our aim is to record single neuron activity in the superficial layers of the monocular portion of the primary visual cortex of juvenile rats (23 days old, p23). We had to reduce the size and weight of the arrays to implant them on small animals without affecting their ability to move freely. We now have a working electrode design (see photo below), but have run into significant problems getting stable recordings from implanted animals, because for the age animals we require the brain is still growing. While we have had some success the yield (number of neurons we can stably record from) has been low. The next step is to try somewhat older animals with slower growth, but still at an age where we know the form of plasticity we are interested in is still expressed to a high degree. Once this problem is solved we can use these electrodes to follow cortical activity over time and determine how this activity adapts during visual deprivation, and determine whether this adaptive, homeostatic plasticity occurs during sleep or wake. Don Katz will be reducing his time on this project, but will continue to serve in an advisory role.

Ex vivo approach:

To supplement the *in vivo* approach to studying the modulation of cortical plasticity by sleep, we are also taking an ex-vivo approach that has been very successful in my lab. In collaboration with Sacha Nelson's lab we have shown that sleep deprivation depresses cortical function as assayed ex vivo in mouse motor cortex. My postdoc Kiran Nataraj has shown that a similar depression of cortical activity occurs in visual cortex; this suggests that sleep deprivation produces a generalized depression of cortical

activity. This could have a profound impact on cortical synaptic plasticity, which depends on activity propagation through cortical networks. The next steps will be to determine the mechanism of this depression, and to ask whether synaptic plasticity is impaired.

Key Research Accomplishments

1. Miniaturization and modification of electrode arrays for young rats in upper cortical layers.
2. Demonstration of generalized depression of cortical activity induced by sleep deprivation in rodents.

Reportable Outcomes

We expect to have an abstract at the upcoming Soc. For Neuroscience meeting on the sleep deprivation work.

Conclusion

We successfully adapted a complex chronically indwelling electrode array design for use in upper layers of visual cortex; we are now overcoming the additional problem of stabilizing recordings in growing brains. Additionally, in collaboration with Sacha Nelson's lab we have made the novel observation that sleep deprivation produces a generalized depression of cortical activity, which could have profound implications for how plasticity is expressed in the sleep deprived state.

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Aim 6: To determine whether or not sleep is required for fear conditioning (Katz).

Aim 6a: sleep-related plasticity of V1 neurons during and following monocular deprivation.
Introduction: This project, done in collaboration with Gina Turrigiano, involves chronic recording of single neurons from the superficial layers of visual cortex in monocularly deprived rats; the aim is to evaluate the impact of sleep deprivation on deprivation-induced plasticity.

Progress: Information on the progress toward the Aims of this project can be found in Dr. Turrigiano's write-up. Briefly, I have trained a postdoc to build and implant a driveable electrode array designed to record from superficial cortical layers, and to isolate single neurons from these arrays in conscious rats. In addition, we have recently (within the last 2 months) developed a method to cleanly remove the *pia mater* from the cortical surface during surgery, thereby virtually eliminating the dimpling issue discussed in Dr. Turrigiano's write-up.

As noted elsewhere, I have pulled back my involvement in this project to focus my efforts more effectively on the project below.

Aim 6b: sleep-related neural oscillations during hippocampal and non-hippocampal learning.
Introduction: This project involves recording multi-site field potential activity during learning and sleep, following hippocampal-dependent and hippocampal-independent tasks. The aim is to determine how and when distributed parts of mammalian neural circuits involved in learning and memory interact during sleep to promote memory consolidation.

Progress and accomplishments: We have examined changes in behavior and sensory coding related to the emergence of sleep-onset-related rhythms in cortex (Fontanini & Katz, 2005; 2006), and have collected preliminary data suggesting that these rhythms interact dynamically with hippocampal rhythms (which are thought to be the source of memory consolidation). On another front, we have produced data demonstrating that hippocampal activity may actually interfere with non-hippocampal (i. e., amygdalar) learning processes (Stone et al., 2005), a finding that leads us to suspect that sleep deprivation itself may actually enhance such learning. We are in the process of testing this hypothesis and expect to have an abstract concerning work for this project for the upcoming Society for Neuroscience conference.

Conclusion: In the next 12 months, it should be possible to run the full experiments in which multi-site recordings are collected during performance of, and sleep episodes following, learning tasks. We now expect that we will be able to pinpoint neural processes that are enhanced by sleep deprivation, as well as those that are impaired by sleep deprivation.

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Aim 7: To examine qualitative and quantitative protein expression changes during sleep and wake

Introduction
Dr. Jeffrey Agar was hired at Brandeis in June 2005 as an Assistant Professor of Chemistry. His research focuses on mass spectrometry, a technique that will augment research on the mechanisms of sleep and complement microarray gene expression and bioinformatics research.

Body
Since this hire six months ago or so, priorities have been to remodel the laboratory space, choose the proper mass spectrometry equipment, and then procure, install, and make functional this equipment. These goals have now been met and the laboratory is now fully operational. A collaboration is being forged between the Agar laboratory and those of the other investigators to study sleep mechanisms. With MALDI imaging capabilities, we are now able to image proteins with 100 micrometer resolution, approaching the size of large cells and certainly making possible the observation of important changes within small brain regions. This approach is particularly well-suited for assaying changes in neuropeptide composition in defined brain nuclei, which accompany sleep-deprivation for example. This is the kind of collaborative experiment we will first undertake, to search for major changes in protein composition in brain regions important for sleep regulation as a function of sleep and wake or as a function of sleep deprivation.

More generally, we now have the capacity to perform the following experiments with our new equipment: 1) MALDI-mass spectral imaging; 2) Two-dimensional protein and peptide separation on-line with MS; 3) In-gel digests to identify proteins from gels; 4) Whole-protein post-translational modification studies; 5) Protein conformational changes by hydrogen/deuterium exchange and on-line digest; 6) Protein binding partners; 7) Quantitation with or without labeling (ICAT, ITRAQ, etc); 8) MALDI and electrospray FTMS for the identification of small molecule structure; 9) Gas phase reactions within the FTMS. We have placed an emphasis on data analysis, and are using the same project management database that HUPO (human proteomics organization) uses to store the entire human proteome.

Key Research Accomplishments:
None.

Reportable Outcomes:
None.

Conclusions:
The mass spectrometry laboratory has a number of unique capabilities and is among the most advanced laboratories in the world. It is anticipated that this advanced technology will be an integral component of research on the mechanisms of sleep at Brandeis.

Aim 8 - Bioinformatics Aim: To assist sleep labs at Brandeis University with analysis of microarray data (Hong)

INTRODUCTION

Dr. Hong is specialized in bioinformatics and computational biology and was hired by Brandeis University in August 2005. He is now building a computational systems biology lab and has hired a PhD student. Within the last half year, the Hong Lab conducted research on: (a) bioliterature mining to facilitate microarray data analysis; and (b) developing image analysis techniques for analyzing high-content screening (HCS) images generated in genome-wide morphological screens of *Drosophila* primary neural cells using RNA interference (RNAi). We anticipate that he and his team will be a major aid to the rest of the sleep community at Brandeis, at a minimum by helping to analyze microarray data.

BODY

(a) Bioliterature Mining

Retrieving knowledge effectively from a large cache of published literature has become increasingly a challenge in biomedical research. For example, high-throughput experimental approaches such as DNA microarray usually yield a large number of hypotheses related to particular biological processes ((Tavazoie, Hughes et al. 1999; Storch, Lipan et al. 2002; Zhou, Kao et al. 2002; Zhou, Kao et al. 2005). To better utilize the limited resources, it is important to prioritize experiments. A common practice is to collect supporting materials from the published literature. With the ever-growing volumes of publications in biomedical research, it becomes increasingly challenging to curate and categorize all available biomedical literature. Computational tools are urgently needed to perform automatic knowledge mining from the massive amount of text in the public databases. The main goal of this project is to develop tools that automatically classify text written in natural language into Gene Ontology (GO) categories. The GO (Ashburner, Ball et al. 2000) terminology is a controlled vocabulary for describing biological processes and functions and is widely accepted by the biomedical research community. The GO terms represent a collection of standardized descriptions of biological processes and functions.

To this end, we applied machine learning techniques to automatically annotating PubMed abstracts with relevant GO terms. These abstracts can subsequently be searched and retrieved by GO terms. Specifically, we were the first to introduce Bayesian networks (BNs) (Pearl 1988) to associate PubMed[1] abstracts with GO terms. Our approach was used to analyze more than 250,000 PubMed abstract which covered six species: *S. cerevisiae*, *C. elegans*, *D. melanogaster*, *M. musculus*, *R. norvegicus*, and *H. sapiens*. Our results show that BNs is capable of capturing the dependence between words, which is important to convey semantic content. We compared BNs with naïve BNs, which is a popular text categorization tool and does not consider the dependence between words. The results showed that BNs significantly outperformed naïve BNs. We have made this research result available to the community by developing the following tools. A web interface for using GO ID to search for PubMed abstracts is available at http://www.bayesiango.com/. An XML-based web service using the Simple Object Access Protocol is available at http://combio.cs.brandeis.edu/Webservices/BayesianGO.asmx. Users can access the web service via GeneNotes, which provides an integrated interface and is available at http://www.GeneNotes.org.

This work will help users retrieve functional information of genes that are identified as a consequence or perhaps even as a cause of different sleep states in the gene expression analyses using

1 http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?db=PubMed

microarrays. A paper was submitted to the Journal of Bioinformatics for review (Hong and Ge under Review).

(b) HCS Image Analysis for Morphological Study of *Drosophila* Primary Neural Cells using RNAi

The goal of the HCS image analysis project is to develop intelligent image analysis techniques/tools for interactively analyzing a huge number of images. This project is collaboration between the Hong Lab at Brandeis University and the Perrimon Lab at Harvard Medical School. The Hong Lab is responsible for develop image analysis techniques while the Perrimon lab is responsible for generating images. We chose to focus on the genome-wide morphological screens of *Drosophila* primary neural cells. In culture, *Drosophila* primary neural cells can differentiate into motor-, sensory neurons and glia (Fredieu and Mahowald 1989). The neurons can form functional synapses and relay action potentials (Rohrbough, O'Dowd et al. 2003). Importantly, they quite faithfully retain gene expression intrinsic to non-dissociated neurons (Fredieu and Mahowald 1989; Cantera, Luer et al. 2002). RNAi is a new method for silencing gene expression and provides causal links between genes and functions through RNAi-induced loss-of-function phenotypes. RNAi high-throughput screening is increasingly used to identify and understand the molecular components and pathways responsible for key cellular process (Eggert, Kiger et al. 2004; Muller, Kuttenkeuler et al. 2005; Pelkmans, Fava et al. 2005; Wheeler, Carpenter et al. 2005). Thus, primary neural culture RNAi screens could offer great potential to identify interesting novel genes that would not otherwise be found using traditional screening methods. The preliminary results include the identification of genes encoding putative receptors, neuropeptides, ion channels, transporters, vesicle trafficking/cycling proteins, cytoskeleton associated proteins, cell adhesion molecules, NA/RNA associated proteins, transcription factors, enzymes, signal transduction molecules, novel proteins, as well as unannotated sequences. About half of the validated candidates have expression within nervous system tissue during embryogenesis. Hence, this study has a great potential to shed new light on the genetic bases of neural systems that are responsible for sleep controls.

HCS generates cellular image data that is rich in information and is becoming a critical new modality of high-throughput data for functional genomics research. The combination of RNAi and HCS (RNAi-HCS) lead to high-throughput image-based studies of cellular phenotypes under various stimulations and systematic RNAi and provide a powerful platform for drug discovery/development (Bertelsen and Sanfridson 2005; Haney 2005). It is anticipated that genome-wide RNAi-HCS will be used to profile the cellular phenotypic characteristics of genes in the near future. The phenotypic profiling data can be combined with other types of biological data (e.g., transcriptional profiling data and protein-protein interaction data) to elucidate the structure of biological networks. For example, we may combine RNAi phenotypic information and transcriptional profiles to discover novel members of the circadian and homeostatic systems, infer the causal relationships between those members, and identify genes controlling neocortical plasticity.

A genome-wide RNAi-HCS screen typically generates tens of thousands of high resolution images. Existing image analysis tools limit the scope of automatic high-content screening to simple analyses on markers and fail to capture the profound aspects of cellular phenotypes regarding cellular behaviors and morphology. Until now, it requires highly trained scientists to spend days and months examining images and produce only a handful of qualitative results. The development of intelligent interactive image analysis tools/systems will significantly increases the exploitation capacity of the RNAi-HCS technology. The Hong lab has successfully developed (a) image processing algorithms to extract quantitative low-level images features such as the number, size, and intensity of APP-GFP aggregates, as well as the morphological characteristics of the neurons including axon outgrowth and branching characteristics; (b) computational models for recognizing images of abnormal neurons; (c) interfaces allowing users to effectively browsing and navigating the image database; and (d) intelligent

interfaces that allow users to query the database using images and learn from users feedbacks. A NIH proposal has been prepared based on the above preliminary results. As two of the sleep labs at Brandeis use Drosophila as a model and also RNAi experiments in S2 cells, we expect that these advances will directly aid their research. Moreover, we suspect that these imaging tools will also aid studies on brain morphology, in different genotypes or even as a function of different physiological states.

REPORTABLE OUTCOMES:

- Funding applied for. NIH PAR-03-106: Innovations in Biomedical Computational Science and Technology. Proposal title: Intelligent Interfaces for Interactively Analyzing High-Throughput Cellular Images.
- Manuscript under review: Hong, P. and H. Ge (under Review). "GO-Based Text Categorization Using Bayesian Networks." Bioinformatics.
- Ph.D. student recruited: Joshua Kresh, Computer Science, Brandeis University.

CONCLUSION:

The Hong Lab has developed bioinformatics tools for bioliterature mining and high-content image analysis. We anticipate our long term research scheme is to systematically study biological networks by integrating heterogeneous biological data including biological sequences, microarray data, proteomics data, and images of cells treated by RNAi and various chemical compounds.

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Conclusion

The sleep consortium at Brandeis is entering a mature phase, where much of the work initiated over the past two years should reach fruition (i.e., publication) over the next year. We also anticipate that the new additions, Drs. Agar and Hong, will greatly aid our efforts, bringing to the group advanced mass spectrometry and bioinformatics tools, respectively.